



## Variation in fat mobilization during early lactation differently affects feed intake, body condition, and lipid and glucose metabolism in high-yielding dairy cows<sup>1</sup>

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### ABSTRACT

Fat mobilization to meet energy requirements during early lactation is inevitable because of insufficient feed intake, but differs greatly among high-yielding dairy cows. Therefore, we studied milk production, feed intake, and body condition as well as metabolic and endocrine changes in high-yielding dairy cows to identify variable strategies in metabolic and endocrine adaptation to overcome postpartum metabolic load attributable to milk production. Cows used in this study varied in fat mobilization around calving, as classified by mean total liver fat concentrations (LFC) postpartum. German Holstein cows ( $n = 27$ ) were studied from dry off until d 63 postpartum in their third lactation. All cows were fed the same total mixed rations ad libitum during the dry period and lactation. Plasma concentrations of metabolites and hormones were measured in blood samples taken at d 56, 28, 15, and 5 before expected calving and at d 1 and once weekly up to d 63 postpartum. Liver biopsies were taken on d 56 and 15 before calving, and on d 1, 14, 28, and 49 postpartum to measure LFC and glycogen concentrations. Cows were grouped accordingly to mean total LFC on d 1, 14, and 28 in high, medium, and low fat-mobilizing cows. Mean LFC ( $\pm$ SEM) differed among groups and were  $351 \pm 14$ ,  $250 \pm 10$ , and  $159 \pm 9$  mg/g of dry matter for high, medium, and low fat-mobilizing cows, respectively, whereas hepatic glycogen concentrations postpartum were the highest in low fat-mobilizing cows. Cows in the low group showed the highest dry matter intake and the least negative energy balance postpartum, but energy-corrected milk yield was similar among groups. The decrease in body weight postpartum was greatest

in high fat-mobilizing cows, but the decrease in backfat thickness was greatest in medium fat-mobilizing cows. Plasma concentrations of nonesterified fatty acids and  $\beta$ -hydroxybutyrate were highest around calving in high fat-mobilizing cows. Plasma triglycerides were highest in the medium group and plasma cholesterol concentrations were lowest in the high group at calving. During early lactation, the decrease in plasma glucose concentrations was greatest in the high group, and plasma insulin concentrations postpartum were highest in the low group. The revised quantitative insulin sensitivity check index values decreased during the transition period and postpartum, and were highest in the medium group. Plasma cortisol concentrations during the transition period and postpartum period and plasma leptin concentrations were highest in the medium group. In conclusion, cows adapted differently to the metabolic load and used variable strategies for homeorhetic regulation of milk production. Differences in fat mobilization were part of these strategies and contributed to the individual adaptation of energy metabolism to milk production.

**Key words:** dairy cow, fat mobilization, energy metabolism

### INTRODUCTION

The energy demand after parturition for maintenance of body tissue function and milk production in high-yielding dairy cows is up to 3 times higher than before calving (Drackley et al., 2001). During early lactation, the extent of nutrient intake is not capable of meeting the requirements of energy because DMI increases only moderately until it peaks, usually several weeks after the highest milk yield (Ingvarsen and Andersen, 2000). Therefore, the transition period and the early-lactation period are characterized by the mobilization of body reserves of different tissues, in particular fat tissue (Ingvarsen and Andersen, 2000; Drackley et al.,

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2001), to cover the energy demand, and include various metabolic and endocrine adaptations. It seems that cows with high genetic merit for milk production mobilize more body reserves than cows with lower genetic merit (McNamara and Hillers, 1986; Pryce et al., 2001), to bridge the gap between energy intake and energy output. High rates of fat mobilization lead to markedly increased plasma concentrations of NEFA and, in consequence, the accumulation of triglycerides (TAG) in the liver (Bobe et al., 2004; Overton and Waldron, 2004). Recent research has suggested considerable variation among high selected dairy cows for releasing energy fuels from stored adipose tissues (Chilliard et al., 2000; Hammon et al., 2009). Tamminga et al. (1997) observed great differences among cows in fat mobilization during the first 8 wk of lactation, ranging from 8 to 57 kg of body fat. Hence, liver fat concentration (LFC) indicates large interindividual variation among cows as a function of the increasing plasma concentration of NEFA from lipolysis in fat depots and fat storage as TAG in the liver. These processes might potentially lead to metabolic disorders that can cause health, welfare, and reproductive problems and occur mainly in the transition period to lactation (Herd, 2000; Ingvarsen and Andersen, 2000). However, high LFC and high precalving body condition do not necessarily lead to diseases related to metabolic disorders (Goff and Horst, 1997; Vernon, 2005). Obviously, some cows are able to cope with metabolic stress very well, whereas others are not (Herd, 2000; Drackley et al., 2001). This ability to deal with metabolic disturbances apparently also depends on important individual cow factors as well as on environmental conditions (Kessel et al., 2008).

It is well established that metabolic adaptation is a complex process, which is also highly variable among cows (Jorritsma et al., 2003; Hammon et al., 2009; van Dorland et al., 2009). Many studies have investigated metabolic changes during the transition period, and how prenatal nutrition and feeding management in early lactation and infectious stressors can affect the metabolism (Grummer, 1993; Drackley et al., 2001; Geelen and Wensing, 2006). In addition, differences between cows for metabolic regulation in the transition period could be explained by parity (van Dorland et al., 2009). Fewer investigations have considered the relevant interindividual variations in fat mobilization and their effect on energy metabolism in association with milk production and body condition. We have recently demonstrated that cows with high and low LFC after calving showed different strategies of nutrient partitioning to provide energy for milk production under similar management conditions. Our hypothesis was that glucose and lipid metabolism are impaired because of high

fat mobilization after calving in dairy cows. The aim of the present study was to identify variable strategies in metabolic and endocrine adaptation related to energy metabolism in high-yielding dairy cows within the same lactation number and held under equal feeding, housing, and management conditions, but differing in LFC.

## MATERIALS AND METHODS

### *Animals, Husbandry, and Classification of Groups*

The experimental procedures were carried out according to the animal care guidelines and were approved by the relevant authorities of the State Mecklenburg-Vorpommern, Germany (LALLF M-V TSD 7721.3-1.1-005/09). German Holstein cows ( $n = 27$ ) with comparable milk production were purchased from 4 local farms at approximately 300 DIM in their second lactation. Cows were then kept in a free-stall barn at the Leibniz Institute for Farm Animal Biology (FBN, Dummerstorf, Germany) to adapt to the new environmental conditions until dry off. From 10 d before until 1 d after parturition, cows were housed in calving boxes. The dry-off period started in wk 8 before expected calving, and cows received intramammary applied antibiotic prophylaxis (Nafpenzal, Intervet, Unterschleißheim, Germany). Cows were studied from wk 8 before expected calving up to 63 DIM in their third lactation. Cows were classified, based on mean LFC on d 1, 14, and 28 after calving, according to the method of Bobe et al. (2004) as low ( $<200$  mg of total fat/g of DM;  $n = 10$ ), medium (200 to 300 mg of total fat/g of DM;  $n = 10$ ), and high ( $>300$  mg of total fat/g of DM tissue;  $n = 7$ ) fat-mobilizing groups. Mean LFC on d 1, 14, and 28 after calving for cows in the high, medium, and low groups were  $351 \pm 14$ ,  $250 \pm 10$ , and  $159 \pm 9$  mg/g of DM of liver tissue, respectively. Five cows originated from farm 1 (2, 1, and 2 for the high, medium, and low groups, respectively); 4 cows were from farm 2 (1, 2, and 1 for the high, medium, and low groups, respectively); 4 cows were from farm 3 (1, 1, and 2 for the high, medium, and low groups, respectively); and 14 cows were from farm 4 (3, 6, and 5 for the high, medium, and low groups, respectively).

### *Feeding, Feed Samples, and Measurement of Zootechnical Data*

Cows were fed with separate TMR ad libitum during the far-off period (wk 8 to 4 before calving), close-up period (wk 3 to 0 before calving), and lactation (wk 1 to 9 after calving), respectively. Ingredients and chemical composition of the different diets are shown in Table 1. The TMR was placed in troughs on scales, which were

**Table 1.** Ingredients and chemical composition of the far-off, close-up, and lactation diets fed during the study on dairy cows

Item	Diet		
	Far-off <sup>1</sup>	Close-up <sup>1</sup>	Lactation
Ingredient			
Corn silage, g/kg of DM		341	287
Grass silage, g/kg of DM	765	290	171
Straw, g/kg of DM	126	102	18
Hay, g/kg of DM	101		45
Concentrate MLF 2000, <sup>2</sup> g/kg of DM			336
Concentrate Universal 18/3, <sup>3</sup> g/kg of DM		179	
Extracted rapeseed meal, g/kg of DM		56	52
Dried beet pulp, g/kg of DM			72
Minerals, g/kg of DM	8 <sup>4</sup>	20 <sup>5</sup>	9 <sup>6</sup>
Propylene glycol, g/kg of DM		12	10
Chemical analysis			
Utilizable protein, <sup>7</sup> g/kg of DM	127	133	164
Crude fat, g/kg of DM	36	34	29
NE <sub>L</sub> , <sup>7</sup> MJ/kg of DM	5.8	6.4	7.1
ADF, g/kg of DM	254	194	166
NDF, g/kg of DM	448	357	299

<sup>1</sup>The far-off period was from wk 8 to 4 before calving, and the close-up period was from wk 3 to 0 before calving.

<sup>2</sup>Concentrate MLF 2000 (Vollkraft, Güstrow, Germany) provided the following: 33% extracted soybean meal, 20% corn, 17% wheat gluten, 13% wheat, 8% extracted rapeseed meal, 5% sugar-beet pulp, 2% sodium hydrogen carbonate, 1.3% calcium carbonate, 0.2% sodium chloride, 8.0 MJ of NE<sub>L</sub>/kg of DM, and 204 g of utilizable protein/kg of DM.

<sup>3</sup>Concentrate Universal 18/3 (Vollkraft) provided the following: 20 to 40% cereals (triticale, rye, wheat, barley), 25% rapeseed expeller, lower contents of malt germs, wheat gluten, wheat bran, peeled oat bran, beet-pulp chips, molasses and glycerin, minerals, vitamins, 6.7 MJ of NE<sub>L</sub>/kg of DM, and 160 g of utilizable protein/kg of DM.

<sup>4</sup>Rinderstolz 9235 (Salvana, Sparrieshoop, Germany) provided the following: 75% crude ash, 4.5% calcium, 6% phosphorus, 10% sodium, 12% magnesium, and vitamins.

<sup>5</sup>Anionen-Mix 1141 (Salvana) provided the following: 14% calcium, 12.5% sulfur, 12% chlorine, 10% magnesium, 2% phosphorus, and vitamins.

<sup>6</sup>Rinderstolz 9522 (Salvana) provided the following: 92% crude ash, 20% calcium, 5% phosphorus, 6% magnesium, 8% sodium, and vitamins.

<sup>7</sup>German Society of Nutrition Physiology (2008).

connected to a computer, and individual feed intake was calculated for each day. Diets were fed at 0600 and 1600 h. The cows had free access to water. Dry matter content was determined weekly from the TMR and grass and corn silages. For determination of DM, feed samples were dried at 60°C for 24 h and subsequently at 105°C for 3 h. Samples from complete TMR were taken monthly, and those from grass and corn silage were taken every 2 wk. Samples were stored at –20°C for determination of nutrient composition at the Institute for Farm Animal Science and Technology (University of Rostock, Germany) and at the Agricultural Analysis and Research Institute (LUFA, Rostock, Germany). Dry matter, CP, ADF, NDF, starch, and sugar were determined according to Naumann and Bassler (2004). The contents of NE<sub>L</sub> and utilizable protein in the diets were calculated according to the German Society of Nutrition Physiology (2008). Energy balance (EB) was calculated as follows: EB = NE<sub>L</sub> intake – (ECM

× 3.14 + 0.293 × kg of BW<sup>0.75</sup>) and was expressed in megajoules per day per cow (Reist et al., 2003).

Body weight was measured after the morning milking twice per week. Backfat thickness (BFT) was determined after the morning milking by ultrasonic measurement (SonoSite Titan; SonoSite GmbH, Erlangen, Germany) once per week during the study in the sacral region according to the method of Schröder and Staufenbiel (2006), and BCS was assigned based on a 1- to 5-point scale according to the method of Edmonson et al. (1989) at the same time.

The cows were milked twice daily at 0600 and 1700 h. Milk yield was recorded electronically after each milking. Milk samples were taken weekly during 1 afternoon and the successive morning milking and were pooled for the determination of protein, fat, lactose, urea, and acetone concentrations in milk. Determination of these milk ingredients was performed by the Landeskontrollverband für Leistungs- und Qualitätsprüfung Mecklen-

burg-Vorpommern e.V. (Güstrow, Germany), using an infrared spectrophotometric method (MilkoScan, Foss Germany, Rellingen, Germany). Energy-corrected milk was calculated as follows: ECM (kg) =  $(0.038 \times \text{g of crude fat} + 0.024 \times \text{g of CP} + 0.017 \times \text{g of lactose}) \times \text{kg of milk}/3.14$  (Reist et al., 2003).

### **Blood Sampling and Analyses of Metabolites and Hormones**

Blood samples were taken on d 56, 28, 15, and 5 before expected calving, 1 d after calving, and then weekly until 63 DIM by jugular vein puncture using tubes (Vacuette; Greiner Bio-One International AG, Frickenhausen, Germany) containing potassium-EDTA ( $\text{K}_3\text{EDTA}$ , 1.8 g/L of blood) as anticoagulant. When the blood sampling and liver biopsy were planned on the same day, blood samples were taken before the liver biopsy. Blood samples were immediately put on ice and then centrifuged at  $1,500 \times g$  at  $4^\circ\text{C}$  for 20 min. The supernatant was harvested and stored at  $-20^\circ\text{C}$  until analyzed for NEFA, BHBA, glucose, cholesterol, TAG, urea, insulin, glucagon, cortisol, and leptin. For determination of plasma concentrations of adrenaline and noradrenaline, the harvested supernatant was immediately frozen in liquid nitrogen and stored at  $-80^\circ\text{C}$  until analyzed. Plasma metabolites (NEFA, BHBA, TAG, glucose, cholesterol, urea) were analyzed by the Klinik für Rinder (Stiftung Tierärztliche Hochschule, Hannover, Germany) using the respective kits: NEFA (no. 434-91795) from Wako Chemicals GmbH (Neuss, Germany); BHBA (no. RB 1008) from Randox Laboratories Ltd. (Crumlin, UK); TAG (no. A11A01640) from Horiba Europe GmbH (Darmstadt, Germany); glucose (no. 553-230) and cholesterol (no. 553-124) from MTI-Diagnostics (Idstein, Germany); and urea (no. LT-UR 0050) from Labor + Technik (E. Lehmann, Austria). Analyses were performed automatically by spectrophotometry (Pentra 400; Axon Laboratory, Reichenbach, Germany). Insulin concentrations were determined by RIA as described by van Dorland et al. (2009). Glucagon concentration was detected by RIA using a kit from Linco (GL-32K; Linco Research, St. Charles, MO; Hammon et al., 2009).

Plasma concentrations of adrenaline and noradrenaline were determined in duplicate by HPLC with electrochemical detection. To inhibit the breakdown of catecholamines in bovine plasma by the enzyme semicarbazide-sensitive amine oxidase (Boomsma et al., 1993), 10  $\mu\text{L}$  of 1 M semicarbazid was added to thawed plasma samples (1 mL). After adding 50  $\mu\text{L}$  of internal standard (500 pg of dihydroxybenzylamine; Recipe Chemicals and Instruments GmbH, Munich, Germany), samples were pipetted into extraction tubes

(Recipe Chemicals and Instruments GmbH) containing 20 mg of aluminum oxide previously activated with 600  $\mu\text{L}$  of 2 M Tris-EDTA buffer (pH 8.7). Samples were vortexed for 10 min and centrifuged at  $800 \times g$  for 1 min at  $4^\circ\text{C}$ . The aluminum oxide with the bound catecholamines was then washed 3 times with 1 mL of 16.5 mM Tris buffer followed by centrifugation ( $800 \times g$ , 1 min at  $4^\circ\text{C}$ ). Elution of catecholamines was achieved by adding 120  $\mu\text{L}$  of 200 mM perchloric acid, vortexed for 30 s, and subsequently centrifuged ( $800 \times g$ , 1 min). Aliquots of 40  $\mu\text{L}$  were injected into the HPLC system (Shimadzu Deutschland, Duisburg, Germany). The mobile phase consisted of 58 mM sodium dihydrogen phosphate buffer, 1.2 mM octanesulfonic acid, 0.3 mM EDTA, 0.2 mM potassium chloride, and 8% methanol at pH 5.6. Separation was performed on a Prontosil C18 AQ column ( $250 \times 4$  mm; Bischoff Analysentechnik, Leonberg, Germany), and electrochemical detection was achieved by an ISAAC cell with a glassy carbon working electrode set at a potential of 600 mV (Axel Semrau, Sprockhövel, Germany). Single-point calibration curves were calculated using peak area versus analyte concentration, and response factors were evaluated for each compound in relation to the internal standard. The intra- and interassay coefficients of variation were 4.5 and 9.4% for adrenaline and 1.8 and 3.0% for noradrenaline.

Plasma cortisol concentrations were analyzed in duplicate using a commercially available solid-phase ELISA kit (DRG Diagnostics, Marburg, Germany) according to the manufacturer's guidelines. Cross-reactivities of the antibody to corticosterone and progesterone were 45 and 9%, respectively, and to any further competing plasma steroids lower than 2%. The assay was validated for use with bovine plasma. The test sensitivity was 3.7 ng/mL, and intra- and interassay coefficients of variation were 4.8 and 6.9%. The plasma concentrations of leptin were measured by the Institute of Physiology, Biochemistry, and Animal Hygiene, Bonn University (Bonn, Germany) and determined by ELISA according to the method described by Sauerwein et al. (2004). The intra- and interassay coefficients of variation were 6.3 and 13.9%, respectively, and the mean recovery was 101.4%.

### **Liver Sampling and Analyses**

Liver biopsy samples were obtained by needle biopsy under local anesthesia on d 56 and 15 before calving, and on d 1, 14, 28, and 49 after calving. After cutting the skin, liver tissue was extracted using a tailor-made biopsy needle (outer diameter of 6 mm). Tissue samples were immediately frozen in liquid nitrogen and stored at  $-80^\circ\text{C}$  until analysis. Tissue was homogenized



under liquid nitrogen. Carbon and nitrogen contents were analyzed by combustion and by elemental analysis using mass spectrometry (EA 1108, Carlo Erba Instruments, Rodano, Italy; Delta S, Finnigan MAT, San Jose, CA). The concentration of total liver fat was calculated according to the following equation: liver fat (% of DM) =  $1.3038 \times C$  (% of DM) -  $4.237 \times N$  (% of DM) -  $0.58 \times \text{glycogen}$  (% of DM) -  $0.5215 \times \text{glucose}$  (% of DM) (Duske et al., 2009). Liver glycogen was determined using a commercial photometric test kit based on amyloglucosidase-catalyzed release of glucose (no. 10207748035; Boehringer Mannheim, Mannheim, Germany; Duske et al., 2009).

### Statistical Analyses

Statistical analyses were done with SAS for Windows, release 9.1.3. (SAS Institute, 2004). For BW and BFT, postcalving differences were calculated from wk 3, 5, and 9 relative to wk 1. The revised quantitative insulin sensitivity check index (**RQUICKI**) was calculated as  $RQUICKI = 1/[\log(\text{glucose, mg/dL}) + \log(\text{insulin, } \mu\text{U/mL}) + \log(\text{NEFA, mmol/L})]$ , as described by Holtenius and Holtenius (2007). Zootechnical data, plasma concentrations of metabolites and hormones, and BW and BFT changes after calving, **RQUICKI**, and LFC were assessed by repeated-measurements ANOVA using the MIXED procedure. The ANOVA model used contained the fixed effects of farm (levels: 1, 2, 3, 4), LFC (levels: high, medium, and low), time (levels: day or week relative to calving, as described in the following), the interaction between LFC and time, the random effect of sire of the cow, and the covariates milk yield during the second lactation, DIM during the second lactation, and dry period length. Effects of farm and the covariate were included in the final model only if significant. Repeated measures on each cow were taken into account by using the repeated statement of the MIXED procedure with an autoregressive residual covariance structure. The levels of the repeated variable time were wk 8 to 1 before calving for the prepartum period, wk 3 before calving until wk 4 after calving for the transition, wk 1 to 9 after calving for the postpartum period, and wk 8 before calving to wk 9 after calving for the entire observation period. Data were analyzed for each observation period, separately.

The least squares means and their standard errors were computed for each fixed effect in the ANOVA model to display the results. Additionally, all group differences of these least squares means were tested using the Tukey-Kramer procedure. Differences with  $P < 0.05$  were considered as significant. The CORR procedure was used to calculate and test Pearson corre-

lations between LFC, BW, and BFT changes and DMI after calving.

## RESULTS

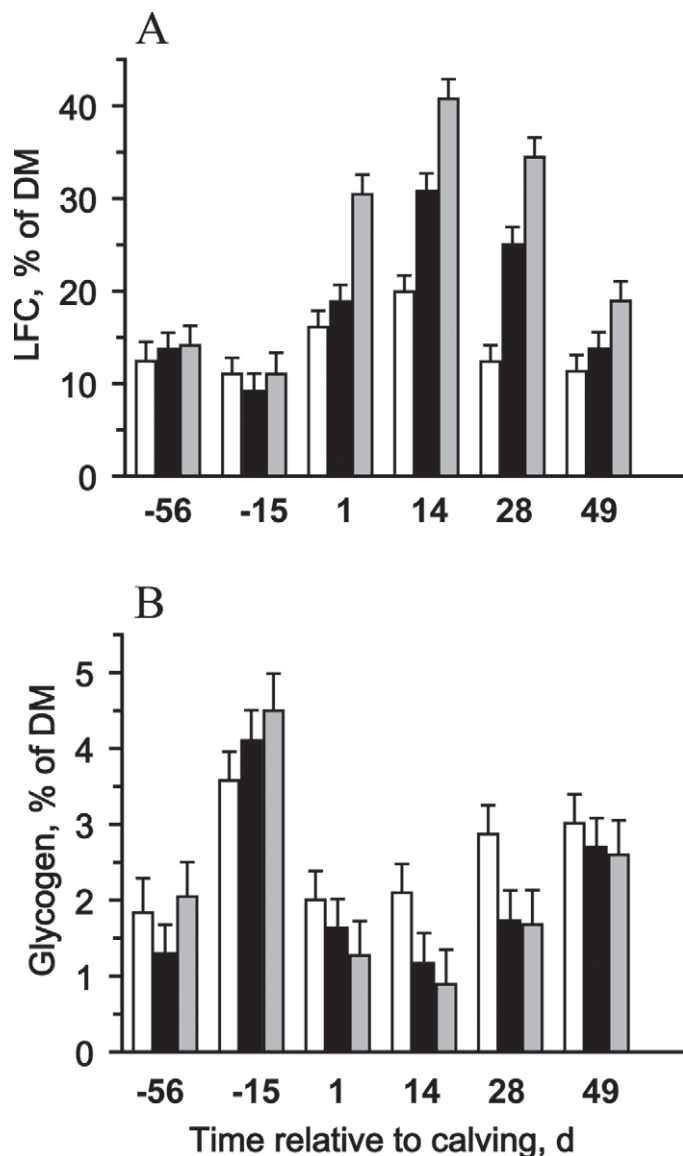
### Production Data Before the Third Lactation and Hepatic Measurements

Milk yield during the second lactation (i.e., in the lactation before the present study) was similar ( $P = 0.6$ ) among groups, with  $11,227 \pm 440$  kg, but the lengths of the second lactation were different ( $P < 0.05$ ), with  $330 \pm 12$ ,  $325 \pm 12$ , and  $375 \pm 14$  DIM for the low, medium, and high groups, respectively. Dry period length did not differ ( $P = 0.3$ ) among groups, with  $56 \pm 4$ ,  $61 \pm 4$ , and  $66 \pm 5$  d for the low, medium, and high groups, respectively.

On d 56 and 15 before parturition, LFC was lower than in the postcalving period and was affected by dry period length and DIM during the second lactation, but did not differ among groups (Figure 1A). After calving, LFC increased ( $P < 0.01$ ) in all groups immediately on d 1 to reach a maximum at d 14 after calving. Postpartum LFC were as classified, that is, highest ( $P < 0.01$ ) in the high group and higher ( $P < 0.01$ ) in the medium group than the low group (Figure 1A). Liver glycogen concentrations followed an inverse course of LFC, and were highest and quite similar among groups before parturition, but decreased rapidly after calving ( $P < 0.05$ ; Figure 1B) in all groups. Postpartum glycogen concentrations in the liver were greater in the low group than in the high ( $P < 0.05$ ) and medium ( $P < 0.1$ ) groups.

### Feed Intake, Milk Production and Composition, and Energy Balance

Dry matter intake decreased ( $P < 0.001$ ) immediately before calving and increased ( $P < 0.001$ ) after calving in all groups (Figure 2A). Dry matter intake prepartum tended to be lower ( $P < 0.1$ ) in the high group than in the low and medium groups, but feed intake was higher ( $P < 0.05$ ) in the low group than in the high and medium groups during the transition and postpartum periods. In addition, farm affected DMI in all investigated time periods. Milk yield increased after parturition ( $P < 0.001$ ) in all cows from  $41.4 \text{ kg} \pm 1 \text{ kg/d}$  in wk 2 up to  $49.1 \pm 1 \text{ kg/d}$  in wk 6, and thereafter did not change much until the end of the experimental period, and without significant group differences (Table 2). Milk yield was influenced by farm ( $P < 0.05$ ) and milk yield during the second lactation ( $P = 0.05$ ). Energy-corrected milk yield increased slightly



**Figure 1.** Total liver fat concentrations (LFC; A) and glycogen concentrations (B) in liver tissue (DM) on d 56 and 15 before calving and on d 1, 14, 28, and 49 DIM in cows with low (white bars), medium (black bars), and high (gray bars) LFC after calving. Data are presented as least squares means  $\pm$  standard errors. Statistically significant ( $P < 0.05$ ) effects for LFC during prepartum (time), transition (LFC, time, LFC  $\times$  time), postpartum (LFC, time, LFC  $\times$  time), and the entire study (LFC, LFC  $\times$  time). Statistically significant ( $P < 0.05$ ) effects for glycogen concentrations during prepartum (time), transition (time), postpartum (LFC, time), and the entire study (time).

up to wk 6 and then decreased ( $P < 0.05$ ) in all groups. Yield of ECM did not differ significantly among groups, but was affected ( $P < 0.05$ ) by farm (Figure 2B and Table 2). Milk fat concentration decreased, but lactose concentration increased ( $P < 0.05$ ) with time (Table 2). The decrease in milk protein concentration ( $P < 0.001$ ) during lactation was more pronounced in the high than the low group (interaction:  $P = 0.05$ ; Figure 2C and

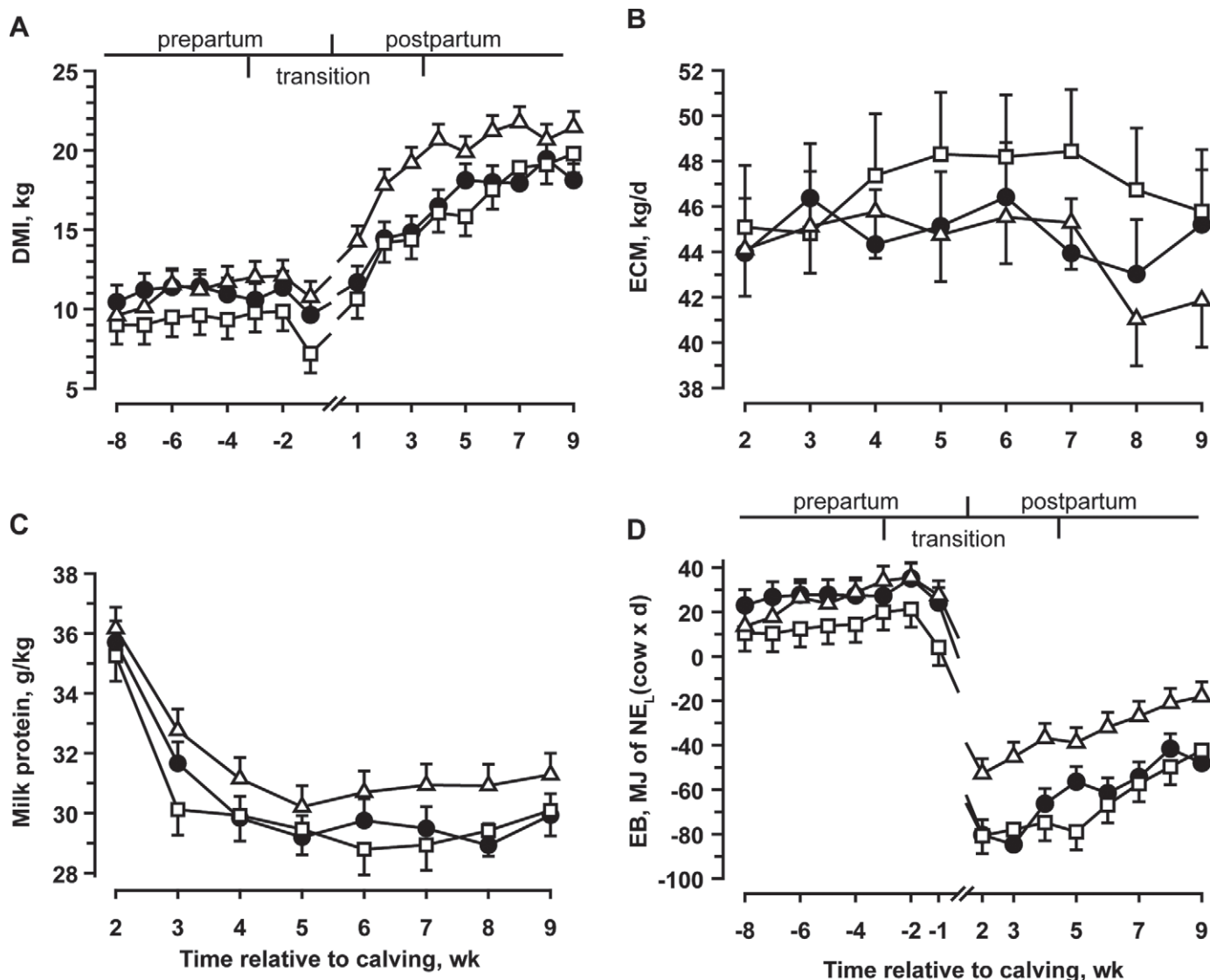
Table 2). The fat-to-protein ratio in milk changed with time ( $P < 0.05$ ) in all groups but did not differ among groups. Milk acetone concentrations were highest in the high group ( $P < 0.01$ ) most notably in early lactation and then decreased in all groups ( $P < 0.001$ ). Milk urea concentrations increased ( $P < 0.001$ ) in all groups, with a trend for the greatest increase in the medium group ( $P < 0.1$ ). The EB decreased ( $P < 0.001$ ) immediately before calving in all groups and was more diminished in the prepartum period ( $P < 0.05$ ) in the high group than in the low and medium groups (Figure 2C). The EB was affected by farm during the dry period ( $P < 0.05$ ). After reaching the nadir in early lactation, EB increased ( $P < 0.01$ ) in all groups and was less negative in the low group than in the high ( $P < 0.01$ ) and medium ( $P < 0.1$ ) groups.

### **BW, BFT, BCS, and Postnatal Changes of BW and BFT**

All groups gained similar amounts of BW ( $P < 0.001$ ) during the last 8 wk of gestation without significant differences (Figure 3A). After calving, BW rapidly decreased ( $P < 0.001$ ) in all groups up to wk 3 in the low group and up to wk 5 in the medium and high groups, and remained constant up to the end of the study. During the transition period, BW tended to be higher ( $P = 0.1$ ) in the low than the medium group. During the entire study, BW was affected ( $P < 0.05$ ) by dry period length. Backfat thickness and BCS increased ( $P < 0.05$ ) before calving in all groups and were highest in the high group ( $P < 0.05$ ; Figure 3B and 3C). After calving, BFT and BCS decreased ( $P < 0.001$ ) in all groups, but the decreases in BFT and BCS were most pronounced in the medium group ( $P < 0.05$ ). The relative decline of BW in wk 3, 5, and 9, compared with wk 1 after calving, was greater ( $P < 0.05$ ) in the high than the low group, whereas the relative decline in BFT was greater ( $P < 0.01$ ) in the medium than the low group (Figure 4). When considering all 27 cows, LFC correlated positively with BW changes ( $r = 0.37$ ;  $P < 0.001$ ), but was not significantly correlated with changes in BFT. Changes in BW were negatively correlated with DIM after calving ( $r = -0.42$ ;  $P < 0.001$ ).

### **Metabolic and Endocrine Changes**

Plasma NEFA concentrations (Figure 5A) decreased ( $P < 0.001$ ) from dry off to d 15 before calving and afterward increased in all groups, showing the highest concentrations on either 7 (low group) or 14 DIM (high and medium groups;  $P < 0.01$ ). Thereafter, plasma NEFA concentrations decreased ( $P < 0.01$ ) in all groups up to 63 DIM. Plasma NEFA concentrations



**Figure 2.** Dry matter intake (A) from wk 8 before calving to wk 9 after calving, ECM (B) and milk protein concentration (C) from wk 2 to 9 after calving, and energy balance (EB; D) from wk 8 before calving to wk 9 after calving in cows with low ( $\Delta$ ), medium ( $\bullet$ ), and high ( $\square$ ) liver fat concentrations (LFC) after calving. Data are presented as least squares means  $\pm$  standard errors. Statistically significant ( $P < 0.05$ ) effects for DMI during prepartum (time), transition (LFC, time), postpartum (LFC, time), and the entire study (LFC, time). Statistically significant ( $P < 0.05$ ) effects for milk protein concentrations during lactation (time). Statistically significant ( $P < 0.05$ ) effects for ECM during lactation (time). Statistically significant ( $P < 0.05$ ) effects for EB during prepartum (LFC, time), transition (LFC, time), postpartum (LFC, time), and the entire study (LFC, time).

during the transition period and after calving were higher ( $P < 0.05$ ) in the high than the low group, and postcalving plasma concentrations were higher ( $P < 0.05$ ) in the high than the medium group. Postcalving plasma NEFA concentrations were affected ( $P < 0.05$ ) by farm. Plasma BHBA concentrations increased ( $P < 0.05$ ) around calving mainly in the high group and were higher during the whole experimental period in the high group than in the low and medium groups (Figure 5B). Plasma BHBA concentrations were affected ( $P < 0.05$ ) by dry period length, showing higher concen-

trations with a shorter dry period length ( $P < 0.05$ ). Plasma glucose concentrations decreased markedly ( $P < 0.05$ ) after calving in the medium and high groups and were higher in the medium group than the high ( $P < 0.05$ ) and low ( $P < 0.1$ ) groups throughout the entire experimental period (Figure 5C). Plasma glucose concentrations were affected ( $P < 0.05$ ) by dry period length and DIM during second lactation. Plasma TAG concentrations decreased ( $P < 0.01$ ) around calving in all groups, and TAG concentrations during transition and after calving were higher ( $P < 0.05$ ) in the medium

**Table 2.** Milk yield, ECM, milk composition, fat-to-protein ratio, milk acetone, and milk urea from wk 2 to 9 in the third lactation of cows with low, medium, and high liver fat concentrations (LFC)

Variable <sup>1</sup>	LFC			SE	ANOVA <i>P</i> -value			Additional effect <sup>2</sup>
	Low	Medium	High		LFC	Time	Time × LFC	
Milk yield, kg/d	45.3	48.0	48.6	1.34	0.2	0.01	0.5	a, b
ECM, kg/d	44.4	46.9	48.9	1.72	0.3	0.05	0.2	a
Milk fat, g/kg	38.8	40.2	41.6	0.15	0.7	0.01	0.8	
Milk protein, g/kg	31.8	30.6	30.3	0.06	0.2	0.01	0.05	
Fat-to-protein ratio	1.22	1.32	1.39	0.05	0.2	0.04	0.8	
Milk lactose, g/kg	48.2	47.8	47.7	0.04	0.8	0.01	0.4	
Milk acetone, mg/kg	1.5	0.9	5.5	0.73	0.01	0.01	0.05	c
Milk urea, mg/kg	177	197	179	7.0	0.3	0.01	0.09	

<sup>1</sup>Values are least squares means with pooled SE.

<sup>2</sup>Significant effect ( $P < 0.05$ ): a = farm; b = milk yield during the second lactation; c = DIM during the second lactation.

than the low group (Table 3). Plasma TAG concentrations were affected by dry period length and DIM in the second lactation. Plasma cholesterol concentration decreased ( $P < 0.001$ ) in all groups from dry off until calving and increased ( $P < 0.001$ ) after calving in all groups, with the highest concentration at the end of the study (Table 3). Plasma cholesterol concentrations were lowest ( $P < 0.05$ ) during transition in the high group. Plasma urea concentration did not differ among groups, but decreased ( $P < 0.001$ ) before calving in all groups and increased steadily ( $P < 0.05$ ) with DIM (Table 3).

Plasma insulin concentrations decreased ( $P < 0.001$ ) and plasma glucagon concentrations increased ( $P < 0.001$ ) with the onset of lactation. Plasma glucagon concentrations tended to be highest ( $P < 0.1$ ) before calving in the low group. Plasma insulin concentrations in the low group were higher ( $P < 0.01$ ) than those in the medium group and tended to be higher than those in the high group after calving (Table 3). Plasma insulin concentrations were affected by milk yield during the second lactation. The glucagon:insulin ratios in plasma increased after calving in all groups and were lower ( $P < 0.05$ ) in the low group than in the medium group after calving (Figure 5D). Data for RQUICKI decreased rapidly at parturition ( $P < 0.01$ ) and increased after calving with DIM. Before parturition, RQUICKI was lower ( $P = 0.05$ ) in the high group than in the medium and low groups, but RQUICKI was higher ( $P < 0.05$ ) during transition in the medium than the high group and postpartum was higher ( $P < 0.05$ ) in the medium group than in the low and high groups (Table 3).

Plasma cortisol concentrations declined from drying off to parturition and showed an excessive increase at calving ( $P < 0.001$ ) in all groups, but decreased ( $P < 0.01$ ) rapidly after calving to prepartum levels. During the transition period, cortisol concentrations were higher ( $P < 0.05$ ) in the medium group than in the high and low groups and postpartum were higher ( $P$

$< 0.05$ ) in the medium than the low group. Cortisol concentrations during transition were affected ( $P < 0.05$ ) by dry period length. Plasma adrenaline and noradrenaline concentrations decreased ( $P < 0.01$ ) during the dry period but increased ( $P < 0.01$ ) immediately before calving in all groups (Table 3). Both hormones decreased slightly ( $P < 0.01$ ) thereafter up to the end of the study. No differences were observed among groups in plasma adrenaline and noradrenaline concentrations.

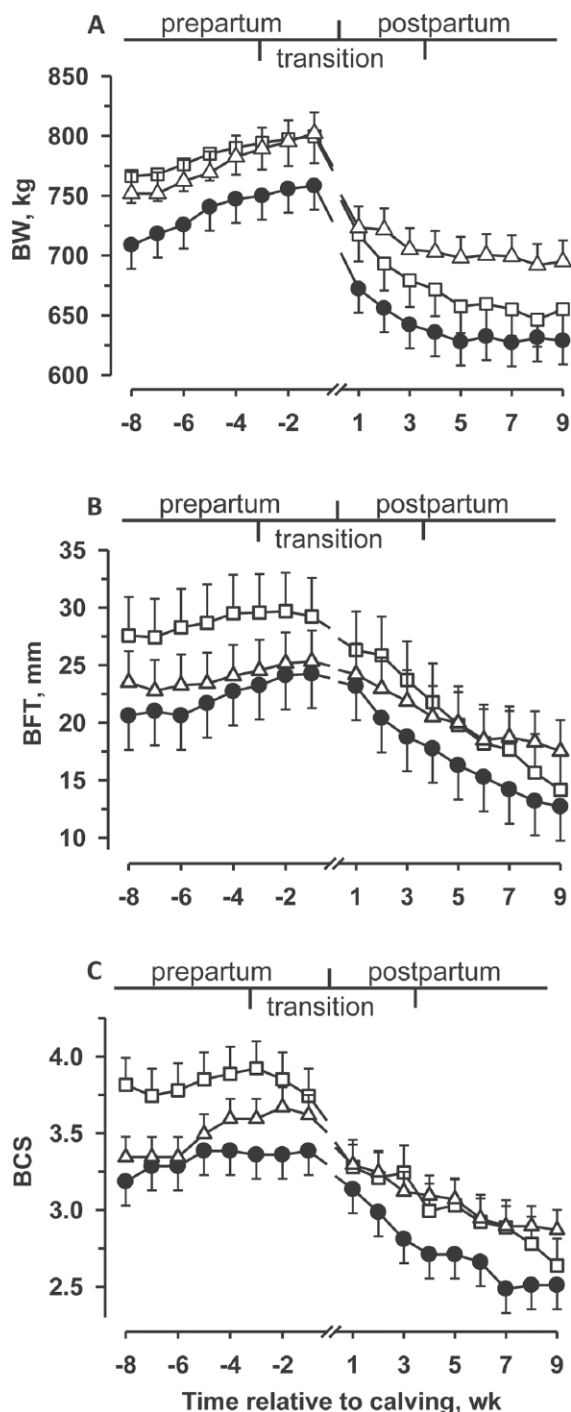
Plasma leptin concentrations increased ( $P < 0.001$ ) from drying off until d -15, and then declined continuously during the first 4 wk of lactation in all groups and remained unchanged afterward. During the entire observation period ( $P < 0.05$ ) and during the transition period ( $P < 0.1$ ), plasma leptin concentrations were higher in the medium than the low group.

## DISCUSSION

### Liver Fat and Glycogen Concentration

Dairy cows investigated in this study were chosen randomly from different farms but had the same lactation number (third lactation) and comparable milk yield during the second lactation. Cows from different farms were evenly distributed within groups. Cows were kept under identical environmental conditions at our institute and were fed the same diets ad libitum. No clinical signs of fatty liver syndrome, such as anorexia, decline in milk yield, or apathy, were observed during the study. On the basis of routinely taken liver samples, we retrospectively grouped cows with respect to total LFC after calving into the high (300 to 400 mg of fat/g of DM liver), medium (200 to 300 mg of fat/g of DM liver), and low (100 to 200 mg of fat/g of DM liver) groups. The variation in fat accumulation in the liver after calving was consistent with findings in the literature (Bobe et al., 2004; Ohgi et al., 2005; Hammon et al., 2009). However, because during the dry-off





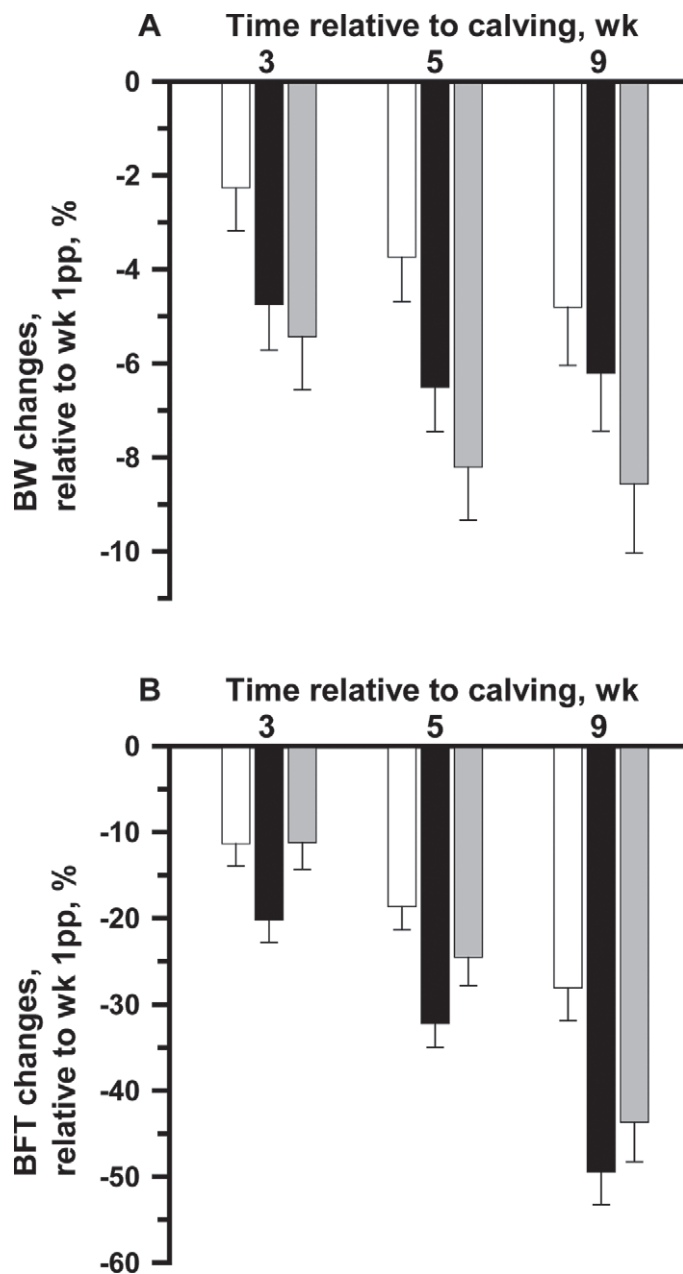
**Figure 3.** Body weight (A), backfat thickness (BFT; B), and BCS (C) from wk 8 before calving to wk 9 after calving in cows with low ( $\Delta$ ), medium ( $\bullet$ ), and high ( $\square$ ) liver fat concentrations (LFC) after calving. Data are presented as least squares means  $\pm$  standard errors. Statistically significant ( $P < 0.05$ ) effects for BW during prepartum (time), transition (time), postpartum (time), and the entire study (time). Statistically significant ( $P < 0.05$ ) effects for BFT during prepartum (LFC, time), transition (time, LFC  $\times$  time), postpartum (time, LFC  $\times$  time), and the entire study (time). Statistically significant ( $P < 0.05$ ) effects for BCS during prepartum (LFC, time), transition (LFC, time, LFC  $\times$  time), postpartum (LFC, time), and the entire study (LFC, time, LFC  $\times$  time).

period LFC was similar among groups, elevated LFC after calving was a result of enhanced uptake of plasma NEFA, whose concentrations increased because of lipid mobilization during the transition period (Bobe et al., 2004). Hepatic glycogen concentrations were inversely related to LFC and were accompanied by decreased plasma glucose concentrations and enhanced glucagon-to-insulin ratios after parturition, as seen by Hammon et al. (2009). Hepatic glycogen concentrations decreased most markedly after calving in the high group, likely because cows in the high group used the most glycogen to compensate for the lowest plasma glucose concentrations at this time, reflecting the low glucose status of cows in the high group (Herd, 2000). This was already observed by Hammon et al. (2009) in cows with high LFC after calving.

#### **Effects of Fat Concentration in the Liver on Milk Production, Feed Intake, Energy Balance, and Body Condition**

Milk yield and milk energy output were equivalent among cows of different LFC, as seen in previous studies (Ohgi et al., 2005; Hammon et al., 2009). All cows began with high milk yield after calving, reached the peak of milk yield in wk 6, and maintained this high level until the end of the observation period. These findings do not confirm that enhanced liver fat accumulation leads to reduced milk production in early lactation, as described by Bobe et al. (2004), but they corroborate our previous study (Hammon et al., 2009), in which comparable milk yields were observed, independent of grouping accordingly to high and low LFC after calving. The observed effects of milk yield during the previous lactation and farm on current milk yield could be explained by slight differences in production level and management at the respective farms. The larger decrease in milk protein content during early lactation for cows in the medium and high groups compared with those in the low group might point at a transient energy deficit for cows in the medium and high groups, which is supported by a more severe negative EB (NEB) in these 2 groups that may have led to reduced milk protein synthesis (Coulon and Remond, 1991; Brun-Lafleur et al., 2010). Milk acetone concentrations were markedly elevated in wk 2 and 3 after calving in the high group compared with the low and medium groups. This finding corresponds to elevated BHBA concentrations in the high group at the same time period, indicating an elevated hepatic oxidation of NEFA to ketone bodies in cows with severe LFC (Grummer, 1993; Bobe et al., 2004; Hammon et al., 2009).

After calving, DMI increased continuously in all groups, and as seen in other studies, the highest DMI



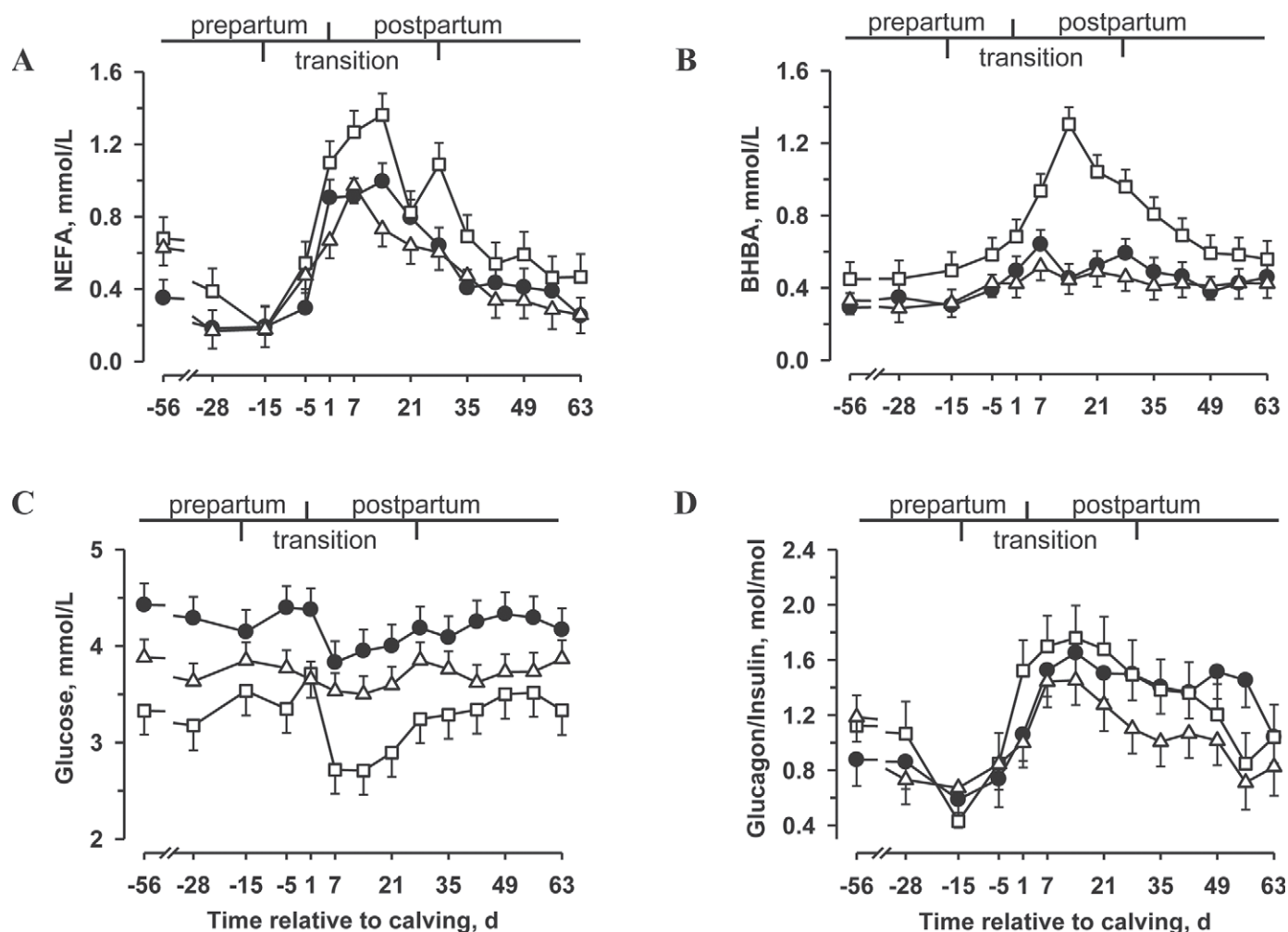
**Figure 4.** Relative changes of BW (A) and backfat thickness (BFT; B) from wk 1 to wk 3, 5, and 9 postpartum (pp) in cows with low (white bars), medium (black bars), and high (gray bars) liver fat concentrations (LFC) after calving. Data are presented as least squares means  $\pm$  standard errors. Statistically significant ( $P < 0.05$ ) effects for changes in BW (LFC, time). Statistically significant ( $P < 0.05$ ) effects for changes in BFT (LFC, time, LFC  $\times$  time).

was observed after the peak of milk yield (Ingvarsen and Andersen, 2000; Grummer et al., 2004). Because of similar milk production among cows, the smaller NEB after calving in the low group was first of all a result of enhanced DMI for cows in the low group (i.e., cows with low LFC and, in addition, low body fat mobilization). Body condition and fat mobilization affect

DMI in high-yielding dairy cows, in agreement with other studies on the interaction of DMI and body fat mobilization during early lactation (Grummer, 1993; Hammon et al., 2009). The negative relationship between BW changes and DMI after parturition supports the effect of fat mobilization on DMI because body fat mobilization postpartum is more or less mirrored by changes in BW. Body reserves and degree of fatness seem to be important factors governing feed intake after calving (McNamara, 2000). However, we also found that optimally conditioned cows (medium group) developed equally severe NEB as cows in the high group because of reduced DMI after parturition. Unexpectedly, cows in the medium group mobilized high amounts of subcutaneous fat but had lower LFC and plasma NEFA and BHBA concentrations than did cows in the high group. Therefore, the demonstrated negative relationship between high BHBA concentrations and low DMI may not hold for all cows (Ingvarsen and Andersen, 2000; Laeger et al., 2010).

As expected, cows in the high group showed the highest BCS, BFT, and BHBA concentrations, but a trend for the lowest DMI already before calving. Body condition may also affect DMI before calving, and precalving body condition is also negatively related to DMI after calving (Kunz et al., 1985; Grummer, 1993; Ingvarsen and Andersen, 2000). Despite a similar body condition for cows in the medium and low groups at calving, cows in the medium group had lower DMI than did cows in the low group postpartum, probably because of greater fat mobilization postpartum for cows in the medium group than the low group. We assume that the degree of fat mobilization after calving might be a more important factor for feed intake regulation than the BFT and BCS before calving. Furthermore, the positive relationship between prepartum and postpartum DMI applied to cows in the high and low groups, in accordance with the report by Grummer (1993), but not to cows in the medium group. Dann et al. (2005) found no relationship between prepartum and postpartum DMI. It is interesting that the reduction of DMI in the last week before parturition (Bertics et al., 1992) was similar in all groups and might not be related to the level of fat mobilization around calving.

On the basis of changes in LFC, BW, and plasma NEFA concentrations around calving, cows in the high group mobilized more body fat than did cows in the medium and low groups, but the decrease in BFT, and therefore mobilization of subcutaneous fat after calving, was greatest for cows in the medium group. Postpartum fat mobilization of subcutaneous fat did not depend on the level of prepartum BCS and BFT, a finding also described by Janovick and Drackley (2010). Obviously, enhanced mobilization of subcutaneous fat



**Figure 5.** Plasma concentrations of NEFA (A), BHBA (B), glucose (C), and glucagon-to-insulin ratio (D) from wk 8 before calving to wk 9 after calving in cows with low ( $\Delta$ ), medium ( $\bullet$ ), and high ( $\square$ ) liver fat concentrations (LFC) after calving. Data are presented as least squares means  $\pm$  standard errors. Statistically significant ( $P < 0.05$ ) effects for NEFA during prepartum (time), transition (LFC, time), postpartum (LFC, time), and the entire study (LFC, time, LFC  $\times$  time). Statistically significant ( $P < 0.05$ ) effects for BHBA during prepartum (LFC, time), transition (LFC, time, LFC  $\times$  time), postpartum (LFC, time, LFC  $\times$  time), and the entire study (LFC, time, LFC  $\times$  time). Statistically significant ( $P < 0.05$ ) effects for glucose during transition (time), postpartum (time), and the entire study (LFC, time). Statistically significant ( $P < 0.05$ ) effects for the glucagon-to-insulin ratio during prepartum (time), transition (time), postpartum (LFC, time), and the entire study (time).

occurs, although prepartum BCS and BFT levels were in an optimal range. Our findings do not support the overall assumption that overconditioned cows mobilize primarily subcutaneous fat depots (Ingvarsen, 2006). In addition, cows in the high group (with the highest LFC) had the highest plasma NEFA concentrations and greatest BW losses after calving, but had lower backfat mobilization than did cows in the medium group. This indicates that adipose depots other than subcutaneous fat were probably used to a greater extent to provide energy substrates for the requirements of maintenance and milk production. Unfortunately, we were not able to measure changes in abdominal fat depots around calving in vivo, but one of the reasons for the differences in NEFA concentrations between cows in the high

group as compared with those in the medium and low groups might be that visceral fat was more readily mobilized in cows in the high group and thus contributed relatively more to elevated LFC and plasma NEFA concentrations for cows in the high group than those in the medium and low groups. Differences in postpartum mobilization of various fat depots were also presumed in a previous study, in which BW and BFT losses after calving showed different time patterns (Hammon et al., 2009). Moreover, when comparing different fat depots in primiparous cows, Akter et al. (2011) found that visceral fat depots were more readily mobilized than subcutaneous fat during the first 15 wk of lactation. Variable changes in the metabolic activity among fat depots are well established in humans (Arner, 1995), in

**Table 3.** Plasma concentrations of triglycerides, cholesterol, urea, insulin, glucagon, revised quantitative insulin sensitivity check index (RQUICKI), cortisol, adrenaline, noradrenaline, and leptin prepartum, during transition, postpartum, and during the entire study in cows with low, medium, and high liver fat concentrations (LFC) after calving

Variable <sup>1</sup>	Time, d	LFC			SE	ANOVA <i>P</i> -value			Additional effect <sup>2</sup>
		Low	Medium	High		LFC	Time	LFC × time	
Triglycerides, mmol/L	Prepartum	0.16	0.14	0.15	0.01	0.5	0.05	0.5	
	Transition	0.09	0.13	0.11	0.01	0.04	0.001	0.7	c
	Postpartum	0.07	0.09	0.08	0.01	0.01	0.001	0.4	b, c
	Entire study	0.08	0.11	0.10	0.01	0.01	0.001	0.5	b, c
Cholesterol, mmol/L	Prepartum	3.07	2.78	2.90	0.21	0.5	0.001	0.2	
	Transition	2.51	2.57	2.12	0.14	0.03	0.001	0.2	
	Postpartum	3.47	3.29	3.42	0.24	0.8	0.001	0.05	
	Entire study	3.29	3.14	3.35	0.22	0.7	0.001	0.01	
Urea, mmol/L	Prepartum	3.54	3.79	3.66	0.18	0.4	0.001	0.5	
	Transition	3.46	3.50	3.33	0.18	0.8	0.01	0.9	
	Postpartum	3.55	3.75	3.51	0.18	0.5	0.001	0.5	
	Entire study	3.54	3.79	3.66	0.18	0.4	0.001	0.5	
Insulin, µg/L	Prepartum	0.59	0.43	0.64	0.13	0.5	0.7	0.6	a
	Transition	0.35	0.20	0.31	0.06	0.2	0.001	0.7	a
	Postpartum	0.32	0.14	0.24	0.04	0.01	0.01	0.8	
	Entire study	0.40	0.22	0.35	0.06	0.05	0.001	0.6	a
Glucagon, ng/L	Prepartum	112	102	97	5.80	0.1	0.001	0.5	
	Transition	111	105	108	5.10	0.6	0.001	0.2	
	Postpartum	120	114	115	5.30	0.4	0.001	0.5	
	Entire study	118	111	110	4.90	0.2	0.001	0.2	
RQUICKI <sup>3</sup>	Prepartum	0.52	0.55	0.48	0.02	0.05	0.16	0.9	
	Transition	0.48	0.51	0.45	0.01	0.03	0.001	1.0	
	Postpartum	0.47	0.52	0.46	0.01	0.01	0.003	1.0	
	Entire study	0.49	0.53	0.46	0.01	0.001	0.001	1.0	
Cortisol, nmol/L	Prepartum	32.80	37.0	39.70	2.40	0.8	0.04	0.4	
	Transition	21.40	44.40	38.10	1.70	0.001	0.001	1.0	b
	Postpartum	19.10	34.80	29.80	1.60	0.03	0.001	1.0	
	Entire study	20.80	37.50	33.40	1.60	0.01	0.001	0.9	
Adrenaline, ng/L	Prepartum	117.0	105.0	130.0	21.5	0.7	0.01	0.3	
	Transition	83.2	97.4	94.5	11.5	0.6	0.001	0.3	
	Postpartum	68.5	80.1	72.3	7.7	0.5	0.2	0.3	
	Entire study	80.4	91.2	83.5	11.7	0.8	0.001	0.4	b
Noradrenaline, ng/L	Prepartum	337	370	367	28.6	0.6	0.04	0.06	
	Transition	323	383	351	24.8	0.4	0.01	0.5	
	Postpartum	312	354	342	25.4	0.4	0.01	0.9	
	Entire study	314	367	350	22.0	0.2	0.01	0.7	
Leptin, µg/L	Prepartum	6.71	7.63	7.27	0.63	0.5	0.001	1.0	
	Transition	5.49	6.77	6.12	0.52	0.09	0.001	0.9	
	Postpartum	5.29	6.33	5.10	0.58	0.2	0.03	0.9	
	Entire study	5.48	6.91	5.89	0.51	0.05	0.001	1.0	

<sup>1</sup>Values are least squares means with pooled SE.

<sup>2</sup>Significant effect ( $P < 0.05$ ): a = milk yield during the second lactation; b = dry period length; c = DIM during the second lactation.

<sup>3</sup>Calculation of RQUICKI =  $1/[\log(\text{glucose}) + \log(\text{insulin}) + \log(\text{NEFA})]$ .

rodents (Wueest et al., 2012), and in ruminants by in vitro studies (Etherton et al., 1977). Our findings that LFC was positively related to BW changes but not to BFT changes after calving indicate that BFT measurements alone are insufficient to predict fat mobilization of all fat depots on an individual cow level. Of course inter- and intramuscular fat may also contribute to the variation in fat mobilization after calving.

#### **Associations Between Liver Fat Concentrations and Metabolites and Hormones**

The higher plasma NEFA and BHBA concentrations for cows in the high group around parturition are in

accordance with other studies (Kokkonen et al., 2005; Ohgi et al., 2005; Hammon et al., 2009) and can be explained by high fat mobilization, resulting in excessive hepatic fat accumulation after calving.

It is interesting that body condition, estimated by BFT and BCS, and fat mobilization were not in accordance with plasma leptin concentrations for cows in the medium group. Thus, plasma leptin concentrations decreased around calving in all groups, as shown before (Reist et al., 2003; Kokkonen et al., 2005), but were highest for cows in the medium group, which exhibited the greatest subcutaneous fat mobilization at the same time. The greater leptin concentration for cows in the



medium group may in part explain the decreased DMI postpartum, in accordance with the results of Ingvarsen and Bosclair (2001). On the other hand, cows in the low group, with the lowest mobilization, had the lowest plasma leptin concentrations, and cows in the high group, with highest the mobilization, had comparable plasma leptin concentrations before calving as those in the medium group but showed the greatest decrease in plasma leptin after calving. Findings by Kokkonen et al. (2005) that BCS is related to plasma leptin cannot be completely confirmed by our study, but our results are in accordance with those of Hammon et al. (2009).

For cows in the high group, the capacity for hepatic fat oxidation was obviously limited. Although for cows in the high group, concentrations of ketone bodies were markedly enhanced, hepatic fat accumulation could not be avoided, in accordance with the results of Litherland et al. (2011). Because hepatic export of fatty acids as TAG within very low density lipoproteins is low in the bovine liver, most of the fat not oxidized to carbon dioxide or ketone bodies is stored as TAG in the liver (Drackley et al., 2001; Bobe et al. 2004). It is interesting that cows in the medium and low groups had comparable NEFA and BHBA concentrations around calving, although these 2 groups differed in LFC and changes in BCS and BFT after calving. It might be speculated whether cows were different in the oxidation of fatty acids to carbon dioxide in the liver and perhaps also in peripheral tissues, such as skeletal muscle, to avoid excessive LFC and hepatic ketone body synthesis. Furthermore, cows may differ in their potential to export hepatic fat by very low density lipoproteins. In this context, it is of interest that cows in the medium group showed the highest plasma TAG concentrations in blood plasma.

The greater glucose demand after parturition for milk production led to decreased plasma glucose concentrations after calving in all cows. Plasma glucose concentrations were lowest for cows in the high group during the entire observation period, but especially around calving. A marked decrease in plasma glucose concentrations in cows with excessive fat mobilization was seen in other studies (Ohgi et al. 2005; Hammon et al., 2009). Lower plasma glucose concentrations after calving were also observed in some studies in which cows were fed fat (Hammon et al., 2008; Duske et al., 2009), but not consistently (Blum et al., 1985). Obviously, more glucose was spared for cows in the high group than in the medium and low groups by using NEFA and BHBA as energy-yielding substrates instead of glucose (Drackley et al., 2001).

The glucagon:insulin ratio tended to be greater for cows in the high than low group, a finding recently associated with elevated gene expression of enzymes

related to hepatic glucose production and also associated with reduced glucose oxidation (Hammon et al., 2009, 2010). The low plasma glucose concentrations may have caused an elevated glucagon:insulin ratio and less glucose utilization in our study. It is interesting that cows in the medium group had higher plasma glucose concentrations but a similar glucagon:insulin ratio postpartum than did cows in the high group. The low plasma insulin concentrations for cows in the medium group may have contributed to the elevated glucose concentrations, but although plasma insulin concentrations were similar for cows in the medium and high groups, those in the medium group had less LFC and a smaller NEFA increase after calving than did cows in the high group. The calculated RQUICKI pointed at greater insulin sensitivity for cows in the medium group than those in the high group. The greater insulin sensitivity of cows in the medium group may have resulted in less body fat mobilization, although subcutaneous fat depots tended to be more reduced for cows in the medium group than those in the high group. These findings again point to differences in the reduction of body fat depots and may indicate differences in insulin responses among different body fat depots. In turn, this would result in variable depletion of body fat among adipose depots, as recently suggested in sheep and mice (Macotela et al., 2009; Lemor et al., 2010). However, further studies will be necessary to clarify insulin responses in different body fat depots in cattle.

Contrary to our findings on insulin sensitivity based on RQUICKI calculation, plasma cortisol concentrations were highest for cows in the medium group. Cortisol is known to cause peripheral insulin resistance and reduced glucose tissue uptake in dairy cows, leading to elevated plasma glucose concentrations, whereas hepatic glucose production is less affected by cortisol (Kusenda et al., 2009; Starke et al., 2009).

For cows in the low group, lower fat mobilization, smaller NEB, and higher DMI went along with elevated higher plasma insulin concentrations postpartum, more so than for cows in the other groups. Therefore, for cows in the low group, higher plasma insulin concentrations reflected the improved energy status postpartum, a relationship noticed previously (Reist et al., 2003). In addition, insulin sensitivity based on RQUICKI calculations was slightly higher for cows in the low group than for those in the high group, especially prepartum, and the plasma glucagon:insulin ratio was lowest for cows in the low group. These findings support the improved energy status postpartum of cows in the low group and support previous findings (Hammon et al., 2009).

It is well established that adrenaline and noradrenaline provoke lipolysis (Etherington et al., 1977; Blum et al., 1982), especially around calving (McNamara and Hill-

ers, 1986; Rukkwamsuk et al., 1998), but we detected no differences in plasma catecholamine concentration among groups. All cows showed increased lipolysis in early lactation, and greater lipolytic activities were associated with elevated adrenaline and noradrenaline concentrations around calving, but plasma concentrations did not reflect the degree of fat mobilization. We suspect that individual fat mobilization might be caused by a different response to catecholamines in adipose tissue (Jaster and Wegner, 1981), the number of adrenergic receptors (Houseknecht et al., 1995), or both.

## CONCLUSIONS

The high LFC of cows in the high group was associated with body fatness at parturition, excessive fat mobilization after calving, reduced DMI in the pre- and postcalving periods, and severe NEB in early lactation. In addition, changes in plasma concentrations of metabolites and hormones around parturition were more severe for cows in the high group, resulting in an impaired glucose homeostasis. The low LFC of cows in the low group were linked with lower fat mobilization, higher DMI, and a small NEB after calving. In addition, cows in the low group showed moderate changes in metabolic traits during the transition period and maintenance of glucose homeostasis. For cows with moderate LFC after parturition (medium group), however, results were ambivalent with regard to changes in feed intake, BCS, and metabolic traits. Although DMI and NEB after parturition were comparable for cows in the high and medium groups, indicating the highest reduction of BFT after parturition, fat mobilization from other fat depots, such as abdominal fat, was apparently less for cows in the medium group than for those in the high group, and the increase in plasma NEFA as well as changes in plasma glucose concentrations during the transition period corresponded to those for cows in the low group. Therefore, the impaired DMI of cows in the medium group after parturition could probably be caused by fat mobilization, but could additionally be a consequence of the action of leptin, because leptin concentrations during the transition period were highest for cows in the medium group. Furthermore, plasma glucose of cows in the medium group may have benefited from low plasma insulin and increased plasma cortisol concentrations, which may have reduced peripheral glucose uptake, but insulin sensitivity based on RQUICKI was highest for cows in the medium group. Because of similar milk production among groups, we conclude that cows in our study adapted differently to the metabolic load and obviously used variable strategies for homeorhetic regulation of energy metabolism

because of milk production. Variable mobilization of different fat depots is probably one key factor in individual adaptation of energy metabolism to milk production, but more in-depth investigations are necessary to understand the diversity of these adaptive processes.

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